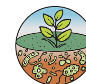
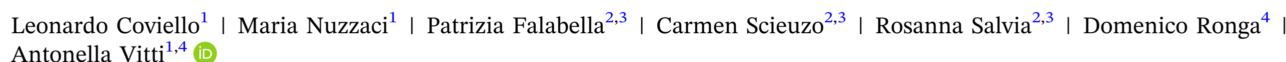
Check for updates

*Journal of Sustainable Agriculture and Environment*




RESEARCH ARTICLE OPEN ACCESS

# Innovative Use of *Hermetia illucens* Frass Extract as Priming to Promote Tomato and Wheat Growth and Protection



Leonardo Coviello[1] | Maria Nuzzaci[1] | Patrizia Falabella[2,3] | Carmen Scieuzo[2,3] | Rosanna Salvia[2,3] | Domenico Ronga[4] | Antonella Vitti[1,4]

[1]Department of Agricultural, Forestry, Food and Environmental Sciences, University of Basilicata, Potenza, Italy | [2]Department of Basic and Applied Sciences, University of Basilicata, Potenza, Italy | [3]Spinoff XFlies s.r.l, University of Basilicata, Potenza, Italy | [4]Pharmacy Department, University of Salerno, Fisciano, Italy

**Correspondence:** Maria Nuzzaci (maria.nuzzaci@unibas.it) | Antonella Vitti (antonella.vitti@unibas.it)

**Received:** 27 September 2024 | **Revised:** 31 October 2024 | **Accepted:** 18 November 2024

**Funding:** This study was carried out within the Agritech National Research Center and received funding from the European Union Next- GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR)—MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4—D.D. 1032 17/06/2022, CN00000022).

**Keywords:** black soldier fly | *Botrytis cinerea* | *Fusarium* spp | superoxide dismutase (SOD) | total phenolic content (TPC) | *Trichoderma harzianum* T22

## ABSTRACT

Frass deriving from *Hermetia illucens* was explored as a new sustainable tool to induce biostimulation and/or antifungal activity in *Solanum lycopersicum* L. var *Cerasiforme* and *Triticum durum* Desf. var *Simeto* against the soil-borne pathogens *Fusarium* spp. Phytotoxicity and in vitro antagonism assessments (mycelial growth inhibition of about 40%) allowed to define the optimal dilution of 10% of pasteurised-frass extract (pFE) to employ for seed priming. Germination tests on water agar demonstrated the priming effectiveness for wheat, but not for tomato, as the analysed parameters were not positively affected. The pFE was used also in combination with the known biocontrol agent *Trichoderma harzianum* T22 (T22), demonstrating that they could work together to obtain a good growth promotion and protective effect in wheat. Indeed, the priming with pFE significantly reduced the disease incidence of almost 60% or 80%, compared to the infected control, if used alone or combined with T22, respectively. The ability of frass extract to control *F. sporotrichioides* in wheat was attributed to both enzymatic and non-enzymatic responses, due to observed differences in total phenolic content (TPC) and superoxide dismutase activity (SOD) in seedlings derived from treated seeds, compared to untreated control. Our findings highlight the great potential of *H. illucens* frass as a sustainable, green, and circular economy-based tool in agricultural systems.

## 1 | Introduction

The world population has increased rapidly in the last century, with the estimation to reach 9.7 billion in 2050 and the consequent worldwide increases of about 70% in food demand (Poveda 2021). The contemporary need to reduce the environmental impact during food production is encouraging researchers to implement useful guidelines for improving seedling quality and sustainability of crops (Ronga et al. 2021). Indeed, the requirement to increase production determined an indiscriminate use of synthetic fertilisers and pesticides in agriculture, resulting in chemical pollution of the natural resources, contamination of food itself and, in turn, dangerous consequences for animal and human health (Lindgren et al. 2018). Therefore, the reduction of the chemical fertilisers and pesticides represents a fundamental objective of current lines of research to contain, or rather reduce, environmental and food pollution (Bargaz et al. 2018).

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2024 The Author(s). *Journal of Sustainable Agriculture and Environment* published by Global Initiative of Sustainable Agriculture and Environment and John Wiley & Sons Australia, Ltd.

Durum wheat (*Triticum durum* Desf., family Poaceae) and tomato (*Solanum lycopersicum* L., family Solanaceae) are widely cultivated crops (Food and Agriculture Organization of United Nations 2023). Climate changes are responsible for a possible increase of illness risk and, therefore, of plant physiological alteration, observed since germination and first seedling growth phases (Vitti et al. 2015). In particular, soil-borne fungi belonging to the *Fusarium* genus are the causal agents of head blight and/or wilt diseases in wheat (Alisaac and Mahlein 2023) and tomato plants (Srinivas et al. 2019). These pathogens affect the seed germination and young seedlings, and they become the limiting factor in their production in both open fields and under greenhouse cultivation systems (Ekwomadu and Mwanza 2023; Iqbal 2024; Nazari et al. 2019; Simões and De Andrade 2023).

Studies carried out with both ancient and modern varieties have provided valuable information on the characteristics of the development of the wheat root system, when a growth promoting microorganism is used (Bochicchio et al. 2022), and its biocontrol effect, when used under biotic stress conditions (Vitti et al. 2022). Indeed, microorganisms, such as *Bacillus* spp. and *Trichoderma* spp., possess the ability to foster plant growth and/or defence against pathogens during the entire plant life cycle, from seed germination to plant maturity (Tsolakidou et al. 2024). In addition to microorganisms, an ever-increasing number of studies is aimed at the identification and optimal use of natural substances that can allow plants to implement adaptation strategies to the main biotic stresses (Rachitha et al. 2017; Godlewska, Ronga, and Michalak 2021), in particular diseases induced by *Fusarium* spp. for wheat and tomato (Chun and Chandrasekaran 2019; Francesconi et al. 2020; Uwineza et al. 2024).

In recent years, insect farming has achieved considerable success for the production of sustainable proteins and secondary products, including frass (Franco 2024). According to current European legislation (European Commission Regulation (EU) No 2021/1925 of 5 November) (European Commission 2021), modifying the Commission Regulation (EU) No 142/2011 (Commission Regulation 2011), frass is «a mixture of excrement derived from farmed insects, the feeding substrate, parts of farmed insects, dead eggs and with a content of dead farmed insects of not more than 5% in volume and not more than 3% in weight». Frass is rich in macro and micro-nutrients and its characteristics could be comparable to those of some types of compost (Arabzadeh et al. 2022). Therefore, insect farming, in particular of bioconverter, and the valorisation of its by-products can be a sustainable strategy for the environment, because it allows not only the by-products disposal and the recovery of nutrients from these matrices, but also reduce the impact of agricultural activities on natural resources by using frass as natural biostimulant/antifungal. Frass in agriculture, according to EC Regulation No 2021/1925 (European Commission 2021), can be used as an organic fertiliser as long as it is subjected to pasteurisation by a heat treatment at 70°C for at least 1 h.

The non-pest fly *Hermetia illucens*, Diptera, Stratiomyidae family, known as the black soldier fly (BSF) is able to convert organic waste into nutrient-rich biomass (Scieuzo et al. 2023). Frass deriving from *H. illucens*, if appropriately diluted, has shown benefits when used for the production of baby-leaf lettuce as a substrate, both in the nursery phase and in the production of potted plants (Setti et al. 2019). Positive effects have been referred also on durum wheat plants grown in pots, in terms of plant height, root dry weight, and chlorophyll indicators (Boudabbous et al. 2023). Good results have been reported also on maize under field conditions: improved growth, yield, and nitrogen use efficiency (Beesigamukama et al. 2020). Recent literature is quite rich about the biostimulant effect of frass on different species (Carroll, Fitzpatrick, and Hodge 2023; Kawasaki et al. 2020; Labella et al. 2024; Romano et al. 2023), meanwhile is rather scarce about its use to protect crops from plant pathogens, as very recently reviewed by Lomonaco et al. 2024. A first study investigated the effect induced from the use of two different concentrations of frass added in soil to verify the effectiveness in controlling soil-borne pathogens of sugar-beet and cress (Elissen et al. 2019). Other studies demonstrated the efficacy of frass to reduce plant death in bean crop or 50% disease rate due to *Fusarium oxysporum* and *Rhizoctonia solani*, respectively (Gebremikael et al. 2020; Quilliam et al. 2020). More recently, Arabzadeh et al. 2022. inquired into the effect of a liquid frass extract, obtained by feeding BSF larvae on a standard Gainesville diet, on five different phytopathogenic fungi (*Alternaria solani*, *Botrytis cinerea*, *Pythium capsici*, *F. oxysporum* and *R. solani*) by an in vitro test, indicating a mycelial growth inhibition of these fungi.

It has been demonstrated that chitinous amendments, as frass is, enhanced the establishment and antagonistic activity of some biocontrol microorganisms, rendering the soil environment more suitable for the success of the introduced biocontrol agents (Barragán-Fonseca et al. 2023). For example, the efficiency of the biocontrol and plant growth promotion by *Bacillus subtilis* AF 1 has been improved by the use of chitin-supplemented formulations (Manjula and Podile 2005). Moreover, the suppressiveness induced by actinobacterial and oxalobacteraceal communities of an experimental agricultural field has been increased when a chitin amendment has been utilised (Cretoiu et al. 2013). More recently, Fuhrmann et al. 2022. indicated that the application of BSF larval residues has influenced the soil microbial communities, particularly fungi.

On these bases, the present work aimed to develop an innovative approach of protection of tomato and wheat plants, based on the use of *H. illucens*-derived frass extract as priming of seeds. For this purpose, a preliminarily possible in vitro antimycotic activity against different fungal phytopathogens and potential phytotoxic/biostimulant effects on test plants was assessed. Finally, frass extract was used to treat the seeds, also in synergistic activity with the known biocontrol *Trichoderma harzianum* T22. Its potential use as a new formulation to ameliorate the germination and seedling growth, and induce a protective effect against *Fusarium* spp. is based on the hypothesis that frass has macro- and micro-nutrients and possible presence of microorganisms producing antifungal compounds responsible to trigger plant resistance by involving enzymatic and/or non-enzymatic defence mechanisms.

## 2 | Materials and Methods

### 2.1 | Plant Material and Fungal Isolates

Wheat (*Triticum durum* Desf. var *Simeto*), tomato (*Solanum lycopersicum* L. var *Cerasiforme*) and cress (*Lepidium sativum* L.



2767035x, 2024, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/sae2.70030 by Patrizia Falabella - University Basilicata Di Potenza Bibl Interdepartim Di Ateneo , Wiley Online Library on [03/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

var *Comune*) seeds were surface sterilised in 1% sodium hypochlorite for 2 min, then rinsed with sterile distilled water thrice and let dry under laminar flow hood.

*Botrytis cinerea* isolate (BC) was provided from the DAFE (Department of Agricultural, Forestry, Food and Environmental Sciences, University of Basilicata) culture collection.

*Fusarium oxysporum* f. sp. *lycopersici* (FOLYC) was kindly provided by Dr. Catello Pane of Consiglio per la Ricerca in Agricoltura e L'analisi dell'Economia Agraria (CREA), Centro di Ricerca Orticoltura e Florovivaismo, Pontecagnano Faiano, Salerno, Italy.

*Fusarium sporotrichioides* PZ2 strain (FS) was kindly provided by Prof. Antonio Ippolito of Department of Soil, Plant and Food Sciences, University “Aldo Moro”, Bari, Italy.

*Trichoderma harzianum*, renamed *T. afroharzianum* (Chaverri et al. 2015), strain T22 (T22), was sourced from *Trianum P* (Koppert Italia S.r.l., Viale delle Nazioni 7, Bussolengo, Verona, Italy).

All fungal mycelia were grown on potato dextrose agar (PDA) for 7 days in the dark at 23°C–26°C.

## 2.2 | *H. illucens* Rearing, Frass Recovery, Chemical and Microbiological Properties

Black soldier fly eggs were provided by Xflies s.r.l. (Potenza, Italy) and stored at 27.0 ± 1.0°C, 70.0% relative humidity (RH) for 48 h. After egg hatching, four groups of 10,000 neonates were reared on the standard Gainesville diet, provided by the animal feed factory Mangimi Losasso s.r.l.—Balvano (Potenza, Italy), consisting of 20% corn, 30% alfalfa meal, and 50% wheat bran (Hogsette 1992), at 70% moisture for 4 days. On the fifth day, each group of 10,000 larvae, the frass and the leftover Gainesville diet were transferred into plastic boxes containing 7 kg of standard diet at 70% of humidity, as reported in Scieuzo et al. 2023. Plastic boxes were incubated in an environmental test chamber (temperature of 27.0 ± 1.0°C, 70.0% RH, photoperiod of 0:24 Light:Dark). The 4-days old larvae were allowed to develop until the stage of prepupae for a total of 10 days. At this stage, the breeding boxes were sieved with a vibrating sieve (Guangzhou Flysource biotechnology Co. Ltd.–China) (5 mm grid size) to separate frass from larvae and prepupae. Frass was pasteurised by a heat treatment at 70°C for 1–2 h, according to the Commission Regulation (EU) No 142/2011 and subsequent modifications of EC Regulation (EU) No 2021/1925 and stored in a hermetic plastic bag until use. Chemical and microbiological characteristics of frass reported in Table S1 were analysed by an external laboratory (ARA, Associazione Regionale Allevatori, Potenza, Italy), according to Labella et al. 2024.

## 2.3 | Frass Extract Preparation

Frass extract was obtained according to Arabzadeh et al. 2022. with minor modification. Briefly, 10 g of pasteurised frass (pF) were added to 100 mL of sterile physiological saline solution (0.5% NaCl) under agitation for 1 h at 27°C. After ultracentrifugation at 6000 × *g* for 15 min, the supernatant was recovered using a double layer of sterile gauze resulting as the final frass extract (pFE).

## 2.4 | Seed Germination Assay With Frass Extract on Different Plant Species

The experiment was performed using cress as the test plant, while tomato and wheat seeds were considered to assess the best pFE concentration to be used in all following experiments. To assess the phytotoxicity of the frass, the extract previously obtained, as such and serial diluted (50%, 25%, 12%, and 6%), was prepared, and the germination test was performed according to Vitti et al. 2024 with little modifications. Briefly, 15 seeds were placed into 90 mm Petri dishes containing sterile filter paper wetted with 5 mL of each dilution and incubated in a growth chamber at 20°C for 72 h (cress and wheat) or 25°C for 96 h (tomato) depending on their different needs for optimal germination. At the end of the incubation time, number of germinated seeds and radicle length (hypocotyl + root) were measured, this latter with ImageJ 1.54 d software (Schneider, Rasband, and Eliceiri 2012).

Seed Germination Index (SGI) was determined according to the following formula:

$$\text{SGI}\ \% = [(\text{n. seeds germinated in sample}/\text{n. seeds germinated in control}) \times (\text{radicle length in sample}/\text{radicle length in control})] \times 100 \quad (1)$$

A value of SGI equal or higher than 80% indicated that frass extract did not induce phytotoxic effect (Luo et al. 2018), while a value significantly higher than 100% indicated a biostimulant effect.

The experiment was carried out in triplicate.

## 2.5 | In Vitro Antifungal Activity

A dual culture overlay assay was used to assess the effect of pFE on mycelial growth (Arabzadeh et al. 2022). Briefly, a first layer of 20 mL of warm PDA (about 50°C), amended or not (control) with 10% frass extract (v/v, pFE/PDA; 1% w/v, pF/PDA), was poured into a 9 cm Petri dish and let incubate in darkness for 48 h at 22°C. A second layer of 10 mL of warm PDA (control) or PDA + pFE was poured on top of the previous layer and let incubate for 24 h at 4°C. After incubation, a 6 mm agar plug from 7 days old fungal PDA mycelial culture of BC, FOLYC or FS was placed in the centre of the plate and incubated for 7 days in the dark at 23°C–26°C. A double layer of only PDA with a central mycelial plug of each fungus represented the control.



2767035x, 2024, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/sae2.70030 by Patrizia Falabella - University Basilicata Di Potenza Bibl Interdepartim Di Ateneo , Wiley Online Library on [03/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Mycelial growth was assessed measuring the average of the three rays (120° from each other) of the colony for each plate. The inhibition percentage of mycelial growth (GI) was assessed by the following formula:

$$GI\% = [(Rc - Rt)/Rc] \times 100, \quad (2)$$

where Rc is the radius of the colonies grown on PDA substrate without pFE and Rt is the radius of the colonies grown on PDA substrate with pFE.

The experiment was carried out in triplicate.

## 2.6 | Effect of Frass Extract on the In Vitro Antagonistic Activity of *T. harzianum* T22 Against Fungal Pathogens

The response of T22 as affected by pFE was evaluated by the dual culture overlay antagonism assay. The plates were prepared as described in the 2.5 section and then the two mycelial plugs (T22 and BC, FOLYC or FS) were placed facing each other at 2.5 cm from the edge of the plate. A double layer of only PDA with a mycelial plug of each single fungus laterally placed represented the control.

GI% was calculated according to the following formula:

$$GI\% = [(Rmc - Rmt)/Rmc] \times 100, \quad (3)$$

where Rmc is the radius of the colonies grown on PDA substrate without T22 and pFE, while Rmt is the radius of the colonies grown on PDA substrate in the direction of the antagonist T22, with or without pFE in the medium.

Plates were incubated for 7 days at 23°C–26°C and the experiment was carried out in triplicate.

## 2.7 | Tomato and Wheat Seed Priming With Frass Extract Alone or Combined With *T. harzianum* and Germination Test on Water Agar

Sterile seeds were soaked for 1 h in sterile distilled water (control), *Fusarium* spp. (FS and FOLYC for wheat and tomato, respectively) spore suspension ($1 \times 10^6$ conidia/mL), *T. harzianum* T22 (T22) spore suspension ($1 \times 10^6$ conidia/mL), 10% pFE in sterile distilled water (v/v), alone or in combination, and then let dry under laminar flow hood till constant weight. The treatments were as follows:

- untreated and healthy control seed (CTRL);
- seed treated with frass extract (pFE);
- seed treated with *T. harzianum* (T22);
- seed treated with frass extract and *T. harzianum* (pFE + T22);
- seed infected with *Fusarium* spp. (FS or FOLYC);
- seed treated with frass extract and infected with *Fusarium* spp. (pFE vs. FS or pFE vs. FOLYC);
- seed treated with *T. harzianum* and infected with *Fusarium* spp. (T22 vs. FS or T22 vs. FOLYC);
- seed treated with frass extract and *T. harzianum*, and infected with *Fusarium* spp. (pFE + T22 vs. FS or pFE + T22 vs. FOLYC).

Twenty mL sterile water agar (WA) (15 g/L) were poured in a 9 cm Petri dish and left to solidify. Ten primed and/or infected wheat or tomato seeds were placed into each Petri dish, and put in a climate control chamber with a photoperiod of 12 h at 23°C (day/night). After 3 days, the plate was covered with a 310 mL volume compostable PLA7 clear cup, according to a modified protocol by Góral and Arseniuk (Góral and Arseniuk 2006). The experiment was carried out in fifteen plates for each condition. At 10 days post sowing, SGI was determined as described in the Section 2.4 on five plates randomly chosen for each condition. Moreover, on wheat seedlings from five plates randomly chosen for each condition, seedling eight, fresh weight of the entire plantlet and dry weight, oven-drying at 60°C until constant weight, were measured.

## 2.8 | Effect of Priming With Frass Extract Alone or Combined With *T. harzianum* in Wheat Against *F. sporotrichioides*

### 2.8.1 | Damping-Off Assessment

The plates were randomly grouped in five groups of three plates for each condition, and the effect of pFE and T22, alone or in combinations, on damping-off caused by *F. sporotrichioides* was evaluated on the emerged seedlings in water agar for each group using the following formula, as described by Veeken et al. 2005:

$$DO\% = [(HPo - HPi)/HPo] \times 100, \quad (4)$$

where HPo is the number of healthy seedlings in the untreated experimental condition and HPi is the number of healthy plantlets in the treated and *Fusarium* spp. inoculated conditions.

### 2.8.2 | Total Phenolic Compound (TPC) Determination

Total phenolic content in wheat tissues (leaf + root) was determined by the Folin-Ciocalteu colorimetric method using catechol as a standard. Tissues (500 mg) of five seedlings randomly chosen from five plates for each condition to obtain five tissue samples for each thesis ($n = 5$) were homogenised in 2.5 mL aqueous methanol 80% (v/v) for 2 h at 25°C, centrifuged at $10{,}000 \times g$ for 5 min and the resulting supernatant was recovered. The reaction was carried out in 96-well plates. Each reaction solution contained 20 µL of methanolic extract previously diluted with distilled water (1:5, v/v) and 100 µL Folin-Ciocalteau reagent previously diluted with distilled water (1:10, v/v). The solution was gently mixed by carefully shaking the plate for a few seconds and left for 3 min. Then, 80 µL of 7.5% sodium carbonate was added and the solution was mixed again. After dark incubation for 2 h, absorbance was measured at 750 nm using a microplate reader (mod. Multiskan FC, Thermo Scientific, Fisher Scientific, Segrate, Italy). The mean absorbance



27679035x, 2024, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/sae2.70030 by Patrizia Falabella - University Basilicata Di Potenza Bibl Interdepartim Di Ateneo , Wiley Online Library on [03/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

value of five sample extracts for each condition, assayed in duplicate, was taken.

#### 2.8.3 | Antioxidant Enzyme Activity

Superoxide dismutase (SOD, EC1.15.1.1) enzyme activity was determined according to Vitti et al. 2016. with some modifications, starting from frozen leaf + root tissues (500 mg) of five seedlings randomly chosen from five plates for each condition, to obtain five tissue samples for each thesis ($n = 5$). Briefly, SOD extraction was carried out homogenising the tissues in 5 mL of cold 20 mM HEPES buffer, pH 7.2, containing 1 mM EGTA, 210 mM mannitol, and 70 mM sucrose, and then centrifuged at 1500 × g for 5 min at 4°C. The resulting supernatant was recovered and absorbance was read at 450 nm using the microplate reader Multiskan FC, according to the manufacturer's instructions (SOD Assay Kit, item No. 706002, Cayman Chemical, Ann Arbour, MI, USA). One unit of SOD was defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical.

The mean absorbance value of five sample extracts for each condition, assayed in duplicate, was taken into account.

### 2.9 | Statistical Analysis

Normal distribution of data was tested by the Shapiro-Wilk test at $p < 0.05$ and homoscedasticity was tested performing the Breusch-Pagan test ($p < 0.05$). Data obtained from in vitro experiments and seed germination test on water agar, indicated as percentages relative to the control (%), damping-off and spectrophotometric data, all expressed as mean ± SD, were analysed according to one- or two-way ANOVA (as specified in each table header and figure caption) followed by Tukey's HSD test ($p < 0.05$).

R (version 4.2.3, R Foundation for Statistical Computing, Vienna, Austria) with the software RStudio IDE (release 2023.06.0 + 421) and packages tidyverse (Wickham et al. 2019) and multcompView (Graves, Piepho, and Selzer 2024), to write and run R code were used.

## 3 | Results

### 3.1 | Frass Extract Phytotoxicity and Biostimulating Properties Assessment

Results from seed germination tests on cress, tomato and wheat are shown in Table 1. A value below 80% of the seed germination index (SGI) indicated a phytotoxic effect (Luo et al. 2018), while a value significantly higher than 100% indicated a biostimulant effect by frass extract. pFE induced a biostimulant effect on all tested species. In particular, the SGI values higher than 100% of control were found at the dilution used of 12% and 25% for cress, in the range 6%–25% for tomato and at 6% and 12% in wheat. This effect was mainly due to the significantly enhanced value of RL in cress and tomato, while a significant increase of both G and RL were observed in wheat.

Frass extract determined a phytotoxic effect only at the highest dilution of 100% in all species, but also at 50% in cress, and at

**TABLE 1** | Effect of frass extract (pFE) as such and at the different dilutions (50%, 25%, 12%, 6%) on seed germination, radicle elongation and germination index of cress (*Lepidium sativum* L. var *Comune*), tomato (*Solanum lycopersicum* L. var. C*erasiforme*) and wheat (*Triticum durum* Desf. var *Simeto*).

| pFE dilution (%) | Parameter | Cress | Tomato | Wheat |
|---|---|---|---|---|
| 6 | G | $92.9 \pm 24.7^{ab}$ | $100.0 \pm 21.7^{a}$ | $131.6 \pm 9.1^{ab}$ |
| 12 | G | $100.0 \pm 12.4^{a}$ | $87.5 \pm 12.5^{a}$ | $163.2 \pm 9.1^{a}$ |
| 25 | G | $92.9 \pm 12.4^{ab}$ | $91.7 \pm 26.0^{a}$ | $115.8 \pm 9.1^{b}$ |
| 50 | G | $57.1 \pm 12.4^{bc}$ | $91.7 \pm 26.0^{a}$ | $110.5 \pm 15.8^{b}$ |
| 100 | G | $35.7 \pm 12.4^{c}$ | $75.0 \pm 21.7^{a}$ | $31.6 \pm 15.8^{c}$ |
| 6 | RL | $82.2 \pm 11.4^{b}$ | $135.1 \pm 31.6^{ab}$ | $115.8 \pm 1.6^{a}$ |
| 12 | RL | $170.8 \pm 9.3^{a}$ | $158.8 \pm 18.3^{a}$ | $116.9 \pm 0.7^{a}$ |
| 25 | RL | $189.4 \pm 7.9^{a}$ | $130.2 \pm 8.1^{ab}$ | $65.5 \pm 1.0^{b}$ |
| 50 | RL | $78.4 \pm 15.3^{b}$ | $99.5 \pm 24.2^{bc}$ | $59.5 \pm 0.9^{c}$ |
| 100 | RL | $76.9 \pm 4.3^{b}$ | $61.3 \pm 9.4^{c}$ | $34.8 \pm 1.1^{d}$ |
| 6 | SGI | $75.2 \pm 18.3^{b}$ | $130.6 \pm 6.8^{a}$ | $152.3 \pm 9.6^{b}$ |
| 12 | SGI | $171.4 \pm 29.0^{a}$ | $137.5 \pm 8.1^{a}$ | $190.7 \pm 11.5^{a}$ |
| 25 | SGI | $176.0 \pm 25.6^{a}$ | $119.9 \pm 36.1^{a}$ | $75.8 \pm 6.7^{c}$ |
| 50 | SGI | $43.6 \pm 4.0^{b}$ | $95.0 \pm 43.2^{ab}$ | $65.7 \pm 9.5^{c}$ |
| 100 | SGI | $27.8 \pm 10.7^{b}$ | $46.8 \pm 18.1^{b}$ | $11.0 \pm 5.5^{d}$ |

*Note:* Data are indicated as percentages relative to the control (%) and expressed as mean ($n = 3$) ± SD. Different letters in the same column per each considered parameter indicate value significantly different according to one-way ANOVA followed by Tukey post hoc test ($p < 0.05$). SGI equal to or higher than 80% indicates the absence of phytotoxic effects (Luo et al. 2018).
Abbreviations: G, germinated seeds; RL, radicle length; SGI, seed germination index.



27673035x, 2024, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/sae2.70030 by Patrizia Falabella - University Basilicata Di Potenza Bibl Interdepartim Di Ateneo , Wiley Online Library on [03/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

25% and 50% in wheat. This negative effect was caused by a reduction of both G and RL, with the exception of tomato, where the number of germinated seeds did not significantly differ among all used pFE dilutions. Results obtained from phytotoxicity test indicated the optimal dilution of 10% pFE to use for all subsequent experiments.

### 3.2 | In Vitro Antifungal Effect of Frass Extract on Mycelial Growth of *B. cinerea*, *F. oxysporum* f. sp. Lycopersici and *F. sporotrichioides*

The effect of frass extract (pFE) added to the growth medium against fungi is shown in Figure 1. A clear reduction of mycelial growth was observed in plates where pFE was added in PDA medium. Frass extract was effective especially against BC, where an inhibition of 94.6% was observed. On the other side, a 42% and 40% mycelial growth reduction against FOLYC and FS were detected, respectively.

### 3.3 | Effect of Frass Extract on In Vitro Antagonistic Activity of *T. harzianum* T22

*T. harzianum* T22 showed the ability to induce a strong inhibition of all tested phytopathogenic fungi (Figure 2) and, in particular, against BC with a GI% of 95% (Figure 2a). Interestingly, the presence of frass extract in the growth medium did not significantly affect the antagonistic in vitro activity of T22, although it was slightly reduced.

### 3.4 | Effect Induced by Frass Extract and *T. harzianum* T22 on Germination of Tomato and Wheat Seed Treated by Priming and Infected by Fusarium Spp

The effects on tomato seed primed with pFE, T22, and pFE + T22, infected or not with FS, are shown in Figure 3. Compared to the control, priming determined a significant increase of 17% of germinated seeds only when performed with T22 spores. Radicle length and SGI were not affected by priming treatment. Therefore, although never resulting in phytotoxic effects and in some cases pFE treatment was able to increase G, RL and SGI values with respect to control and/or FOLYC, this increase was not significant compared to both control and FOLYC.

The effects on wheat seed primed with pFE, T22, and pFE + T22, infected or not with FS are shown in Figures S1, 4 and 5. Seed priming with pFE, T22 and pFE + T22 never resulted phytotoxic because SGI was always over 80% (Luo et al. 2018). In addition, when used alone, pFE determined a significant biostimulant effect in terms of radicle elongation (RL increase of 33% compared to the 100% of control) and, as a consequence, of SGI, that increased of 44% with respect to not treated control (Figure 4). Only when used together with T22, pFE determined a significant increase in seedling height (+17% on control, data not shown) and in radicle length (+26% on control), but not in SGI due to the significantly lower number of germinated seeds and seedlings obtained, accordingly (Figures S1 and 4). The positive effect induced by pFE on RL was also observed in presence of the pathogen (pFE vs. FS), while the effect was positive in terms of G when it was used with T22 (pFE + T22 vs. FS) (Figures S1 and 4). Indeed, an augmentation of 31% in RL and 17% in G was gained on the control in pFE versus FS and pFE + T22 versus FS, respectively, with an increase of SGI of +36% in the latter. On the contrary, no significant difference was observed in terms of G, RL and SGI in all treated and infected seeds compared to the only infected seeds (FS) (Figure 4).

With regard to weights, as shown in Figure 5, seed priming with pFE + T22 increased fresh weight (FW) by 63% on the control, while in all other treatments no significant differences were denoted. Seed priming with pFE, T22, both alone or combined (pFE + T22), caused an increase in dry weight (DW) of +42%, +28%, +58%, respectively, compared to control. Also in presence of the disease, seedlings derived from seeds treated with pFE and T22 (pFE + T22 vs. FS) showed an increased DW of 44% on the control (Figure 5).

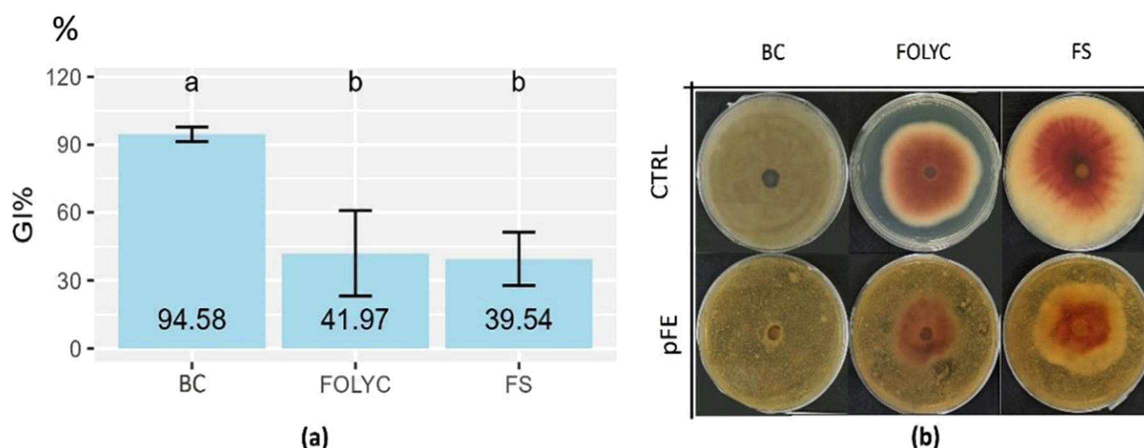



**FIGURE 1** | Mycelial growth inhibition of *B. cinerea* (BC), *F. oxysporum* f. sp. *lycopersici* (FOLYC) and *F. sporotrichioides* (FS) induced by 10% frass extract (pFE) added in the growth media: (a) bars represent the percentage of fungal growth inhibition (GI%). Different letters indicate mean values (n = 3) ± SD significantly different according to one-way ANOVA followed by Tukey post-hoc test ($p < 0.05$); (b) representative images of fungi grown on PDA (CTRL) or PDA + pFE medium (pFE).



27670035x, 2024, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/sae2.70030 by Patrizia Falabella - University Basilicata Di Potenza Bibl Interdepartm Di Ateneo , Wiley Online Library on [03/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

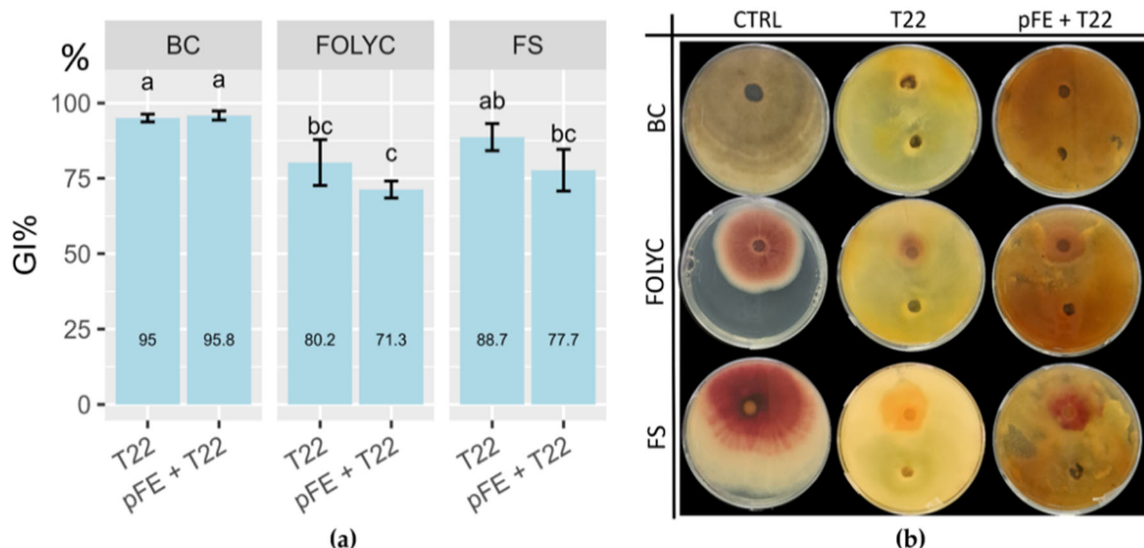



**FIGURE 2** | Mycelial growth inhibition of *B. cinerea* (BC), *F. oxysporum* f. sp. *lycopersici* (FOLYC) and *F. sporotrichioides* (FS) induced by *T. harzianum* T22 (T22) with 10% frass extract added in the growth media (pFE + T22): (a) bars represent the percentage of fungal growth inhibition (GI%). Different letters indicate mean values ($n = 3$) ± SD significantly different according to two-way ANOVA followed by Tukey post-hoc test ($p < 0.05$); (b) representative images of fungi grown on PDA (CTRL), T22 against phytopathogenic fungi on PDA (T22), and T22 against phytopathogenic fungi on PDA + pFE (pFE + T22).

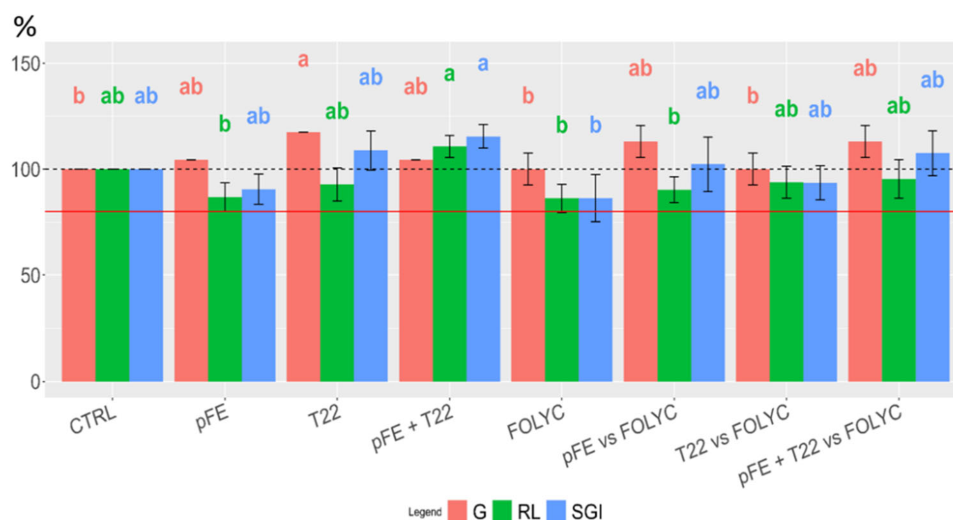



**FIGURE 3** | Germination of tomato (*Solanum lycopersicum* L. var *Cerasiforme*) seeds primed with 10% frass extract (pFE), *T. harzianum* T22 (T22), alone or together (pFE + T22) and infected with *F. oxysporum* f. sp. *lycopersici* (FOLYC) or not. Bars represent the percentage relative to the control (%) of germinated seeds (G, red colour), radicle length (RL, green colour) and seed germination index (SGI, blue colour). For each considered parameter, different letters (same colour) indicate mean values ($n = 5$) ± SD significantly different according to one-way ANOVA followed by Tukey post hoc test ($p < 0.05$). The red line and the dashed black line designate the percentages of the phytotoxicity (80%) and the control (100%) threshold, respectively.

### 3.5 | Protective Effect Against Fusarium Sporotrichioides Induced by Frass Extract and *T. harzianum* T22 on Wheat Seed Treated by Priming

The infection with pathogen spores did not cause presence of symptoms on the seedling aerial parts. On the contrary, the presence of fungal mycelium was clearly observed on seeds and roots (Figure S1c), reisolated on PDA and observed under optical microscope (data not shown). In particular, as reported in Table 2, FS caused an incidence of 48%, while the priming with pFE, T22 or pFE + T22 were able to significantly control the disease. Indeed, pFE alone reduced incidence to 21%, T22 to 4% and pFE + T22 to 11%, indicating the ability of frass extract to control *F. sporotrichioides* almost 60% compared to the positive control (FS) if used alone (pFE vs. FS) and really almost 80% when used together with T22 (pFE + T22 vs. FS).



2767035x, 2024, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/sae2.70030 by Patrizia Falabella - University Basilicata Di Potenza Bibl Interdepartim Di Ateneo , Wiley Online Library on [03/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

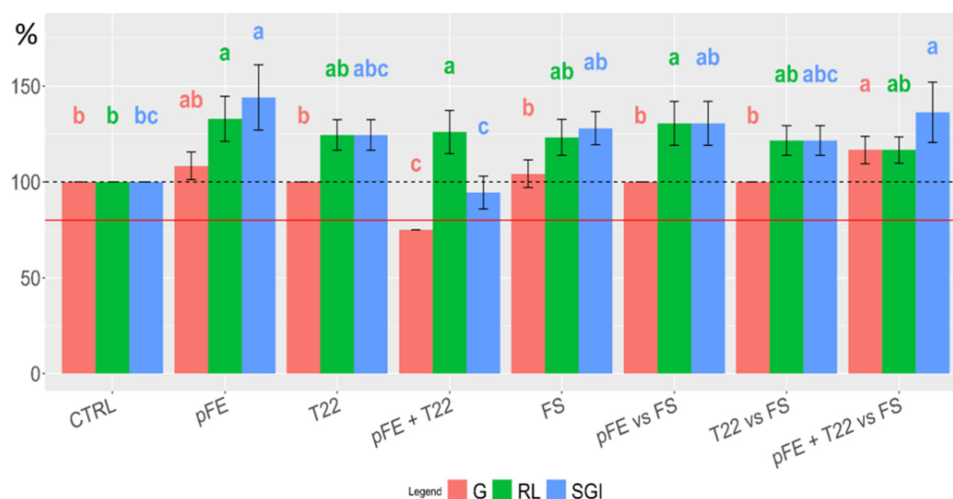



**FIGURE 4** | Germination of durum wheat (*Triticum durum* Desf. var *Simeto*) seeds primed with 10% frass extract (pFE), *T. harzianum* T22 (T22), alone or together (pFE + T22) and infected with *F. sporotrichioides* (FS) or not. Bars represent the percentage relative to the control (%) of germinated seeds (G, red colour), radicle length (RL, green colour) and seed germination index (SGI, blue colour). For each considered parameter (same colour), different letters indicate mean values ($n = 5$) ± SD significantly different according to one-way ANOVA followed by Tukey post hoc test ($p < 0.05$). The red line and the dashed black line designate the percentages of the phytotoxicity (80%) and the control (100%) threshold, respectively.

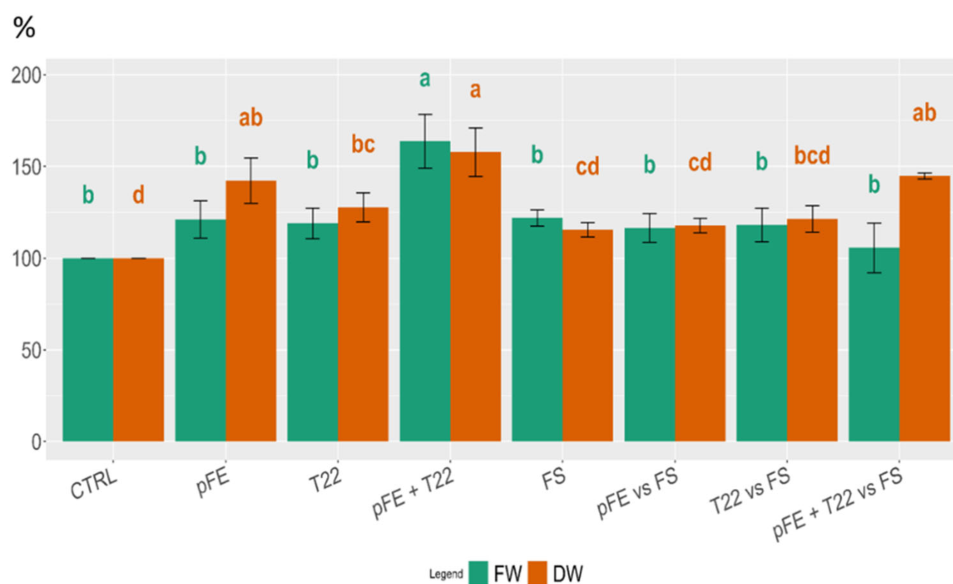



**FIGURE 5** | Seedling growth parameters of durum wheat (*Triticum durum* Desf. var *Simeto*) untreated seed (CTRL) or seeds primed with 10% frass extract (pFE), *T. harzianum* T22 (T22), alone or together (pFE + T22) and infected with *F. sporotrichioides* (FS) or not. Bars represent the percentage relative to the control (%) of fresh weight (FW, green colour), dry weight (DW, red colour). For each considered parameter (same colour) different letters indicate mean values ($n = 5$) ± SD significantly different according to one-way ANOVA followed by Tukey post hoc test ($p < 0.05$).

### 3.6 | Effect of Frass Extract and *T. harzianum* T22 Applied as Seed Priming on Phenolic Compounds and SOD Enzyme Activity in *F. sporotrichioides* Infected Seedlings

The responses of seed infection and priming with frass extract and/or *T. harzianum* T22 was evaluated by quantifying the content of total phenols (TPC) and total SOD activity, respectively, as reported in Figure 6. The highest significant TPC was in tissues of seedlings derived from seeds only infected with the fungus (FS), while the priming induced values of TPC that were never significantly different to control (Figure 6a). On the contrary, SOD activity resulted the highest in control seedlings and significantly lower than CTRL in all the other thesis, in particular when T22 was used for biopriming, with a decrease of −22, −19, −35, and −40% in FS, pFE versus FS, T22 versus FS, and pFE + T22 versus FS, respectively (Figure 6b).



27670354, 2024, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/sae2.70030 by Patrizia Falabella - University Basilicata Di Potenza Bibl Interdepartim Di Ateneo , Wiley Online Library on [03/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

## 4 | Discussion

*H. illucens* is commonly used as a bioconverter insect of organic waste during its larval stage, to dispose of this waste and obtain secondary products of high value. Among the most promising outputs there are chitin and its derivative and frass, which can foster the circularity of the agri-food sector (Scieuzo et al. 2023; Surendra et al. 2020; Triunfo et al. 2022). Frass can be valorised as a sustainable alternative to chemical fertilisers thanks to its known richness in macro- and micro-nutrients (Beesigamukama et al. 2020). Taking into consideration the antimicrobial effects of frass against some phytopathogenic fungi, as very recently reviewed by Lomonaco et al. 2024, in the current study a 10% frass extract (w/v) was employed as an eco-friendly antifungal and biostimulant substance. Frass extract was obtained from thermal treated frass of *H. illucens* larvae reared on the Gainesville diet under standard conditions. Extraction process involved only the use of a physiologic saline solution, resulting in a totally eco-healt-friendly process. Frass extract (pFE) was used in vitro, added in the growth medium, to gain knowledge about the optimal conditions of its use. Subsequently, pFE was employed as an innovative approach for frass application, that is seed priming, to evaluate its ability to stimulate germination and seedling growth and, at the same time, to control the disease induced by the two phytopathogenic fungi *Fusarium oxysporum* f. sp. *lycopersici* and *F. sporotrichioides*, destructive for the two important crops tomato and wheat, respectively.

**TABLE 2** | Percentage of *F. sporotrichioides* (FS) incidence in wheat (*Triticum durum* Desf. var *Simeto*) seedlings derived from seeds infected with FS and primed with 10% frass extract (pFE vs. FS), *T. harzianum* T22 (T22 vs. FS), and pFE and T22 together (pFE + T22 vs. FS), germinated on water agar substrate.

| Treatment | Incidence (%) |
|---|---|
| FS | $47.6 \pm 6.9^{a}$ |
| pFE versus FS | $20.5 \pm 5.6^{b}$ |
| T22 versus FS | $3.7 \pm 6.4^{c}$ |
| pFE + T22 versus FS | $10.7 \pm 0.6^{bc}$ |

*Note:* Different letters indicate mean values ($n = 5$) ± SD significantly different according to one-way ANOVA followed by Tukey post-hoc test ($p < 0.05$).

Frass extract induced a biostimulation effect at dilutions of 12%–25% in cress, used as test plant, between 6% and 25% or 6% and 12% in tomato and wheat, respectively, with a seed germination index (SGI) value always significantly higher than control (see Table 1). It was demonstrated that high electrical conductivity (EC) and salinity reduce the germination and the emergence speed in cress (Dawd and Abdulla 2020; YILDIRIM et al. 2022), as observed in the current study, where percentages no higher than 25% were effective in stimulating the germination of cress seed. In addition, our finding confirms those obtained by Bohm et al. 2023 on *Lactuca sativa* and *Raphanus sativus*. Indeed, authors observed that a 25% dilution of a frass extract derived from BSF-based bioconversion of biosolids led to a subsequent reduction of some toxic compounds, compared to no diluted frass extract. This has been attributed to a decrease of ammonium nitrogen ($NH_4^+$-N) and EC, both negatively correlated to germination. The EC and $NH_4^+$-N values of the different frass used by Bohm et al. 2023 were on average about 2.4 mS/cm and 4.7 g/kg, respectively. In our case the EC is almost two-fold (4.56 ± 0.00 mS/cm) that of Bohm et al. 2023, while $NH_4^+$-N is very similar (4.77 ± 0.51 g/kg) (see Table S1). The higher value of EC could explain the necessity to use pFE at a higher dilution than 25% to obtain the best seeds germination, in particular for wheat. Indeed, pFE always resulted phytotoxic, with SGI values below 80% (Luo et al. 2018), when used as it is, or diluted at 50% in cress, and also at 25% on wheat. In this latter species this can be probably due to wheat germination, that is the phenological phase in which it manifests its highest sensibility to salinity (Francois et al. 1986). On the other hand, tomato seeds resulted less sensitive to EC than wheat, probably because first growth stages of tomato are negatively affected only by EC above 3 mS/cm (Lu et al. 2022), while the pFE dilution of 50% has clearly led to lower values.

Regarding the suppressive effect of frass extract, the inclusion of pFE in the growth medium in the in vitro trial led to a clear inhibition of about 95%, 42% and 40% of the three tested fungi *B. cinerea* (BC), *F. oxysporum* f. sp. *lycopersici* (FOLYC) and *F. sporotrichioides* (FS), respectively (see Figure 1).

The presence of some beneficial microorganisms in a not-filtered frass derived by BSFL reared on Gainesville diet under standard

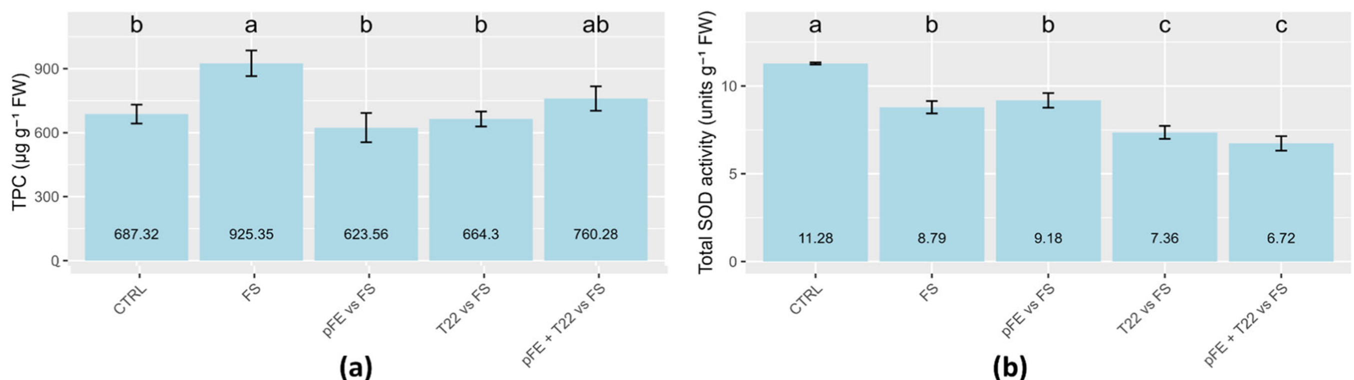



**FIGURE 6** | Total phenol content (TPC) (a) and activity of total superoxide dismutase (SOD) (b) measured in leaf + root tissues of durum wheat (*Triticum durum* Desf. var *Simeto*) of seedlings derived from seeds untreated (CTRL) or infected with *F. sporotrichioides* (FS) and primed with 10% frass extract (pFE vs. FS), *T. harzianum* T22 (T22 vs. FS), and pFE and T22 together (pFE + T22 vs. FS), germinated on water agar substrate. Bars indicate mean values ($n = 5$) ± SD significantly different according to one-way ANOVA followed by Tukey post hoc test ($p < 0.05$).



2767035x, 2024, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/sae2.70030 by Patrizia Falabella - University Basilicata Di Potenza Bibl Interdepartim Di Ateneo , Wiley Online Library on [03/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

conditions, allowed a 95% and 100% inhibition of *B. cinerea* and *F. oxysporum* mycelial growth, respectively (Arabzadeh et al. 2022). At the same time, this frass extract, when microfiltered (0.22 μm), induced a definitely lower inhibition (29%) of the fungal mycelial growth of only *B. cinerea*. The authors supposed that in this case, although microorganisms are removed by filtration process, they were able to produce antifungal compounds, that remain active in the microfiltered frass extract in a concentration high enough to inhibit *B. cinerea* growth, but not that of *F. oxysporum*. Our results showed a 65% higher efficacy against *B. cinerea* and about 40% against *Fusarium* spp. compared to microfiltered frass extract by Arabzadeh et al. 2022. This work is the first to assess the effectiveness of an extract obtained from thermally treated frass against fungal plant pathogens. According to EU regulation, indeed, frass must be treated at 70°C for at least an hour before commercialisation to reduce its content in harmful microorganisms. This treatment could not kill all microorganisms, as already highlighted by Labella et al. 2024, where in a thermally treated frass the bacterium *Escherichia coli* was detected. This can be due to spore-forming bacteria, that can endure extreme conditions, such as high temperatures, and allow them to survive and eventually recolonise substrates once favourable conditions return (Setlow 2016). Our in vitro results suggested that the use of pasteurised frass as extract formulation could be effectively used in controlling fungal plant pathogens, probably due to the presence of microorganisms able to produce antifungal compounds at high concentration with antibiosis effect against different plant pathogens (Arabzadeh et al. 2022).

Several soil microorganisms possess antagonistic activity to limit soil-borne plant pathogens, including *Fusarium* spp. Among them, *Trichoderma* spp. are the most efficacious (Vitti et al. 2022; Kubiak et al. 2023), even to limit the production of mycotoxin accumulation in cereal grain caused by *F. sporotrichioides* (Wiśniewska et al. 2011). On these basis, in the current study the effect of pFE in presence of *T. harzianum* T22 was assessed. *Trichoderma* spp. can produce antibiotics, compete for space and essential nutrients, produce cell-wall degrading enzymes, and induce plant defence mechanisms, determining the suppression of the pathogens (Sood et al. 2020). Based on in vitro analysis, Sempere Ferre and Santamarina (Sempere Ferre and Santamarina 2010) demonstrated that *T. harzianum* is able to compete for space and nutrients with *F. culmorum*, colonising the available surface faster than the pathogen at two different temperatures of growth, 15°C and 25°C. Geng et al. 2022 reported that *T. harzianum* completely inhibits the growth at 25°C of *B. cinerea* on PDA in a dual-culture assay. Similarly to these studies, our results showed that T22 was capable of effectively reducing the mycelial growth of all three considered fungal pathogens, reaching the best performances against *B. cinerea* with a percentage of GI of 95% (see Figure 2). Noteworthy, frass extract added in the growth medium together with T22 did not reduce the inhibition of all considered fungal pathogens in vitro, suggesting that frass extract did not interfere with *T. harzianum* T22 antagonistic activity against BC, as well as FOLYC and FS, which had never studied before.

Regarding subsequent in vivo study, here we focused on seed priming techniques with both pFE and T22, to protect tomato and wheat against FOLYC and FS, respectively. Our results invite further studies on possible use of frass extract, also in combination with *T. harzianum*, in a different approach for plant treatment, to control *B. cinerea*.

Seed priming is a promising, efficient and low cost approach to enhance germinative, growth and productive abilities of crops. Indeed, the seed germination process initiates without radicle protrusion thanks to its controlled imbibition in a solution at low osmotic potential or just in water (Sher 2019). In this work, due to frass richness in macro- and micro-nutrients and possible presence of microorganisms (Arabzadeh et al. 2022; Setti et al. 2019; Labella et al. 2024) we formulated a nutrient+bio-priming with pFE for the seed treatment (Waqas et al. 2019; Mahmood and Kataoka 2019) and evaluated its efficacy when used alone or combined with T22, a known biocontrol agent even used in seed biopriming (Chandra Nayaka et al. 2010; Ferrigo et al. 2020; Sui et al. 2022).

On tomato, priming determined a significant increase of 17% of germinated seeds only when performed with T22. Meanwhile, radicle length and SGI were never affected by priming treatment with pFE (see Figure 3). On the contrary, pFE determined a significant biostimulant effect, above all in terms of radicle elongation in wheat, inducing also a higher number of germinated infected seeds when used together with T22, (pFE + T22 vs. FS) (see Figure 4). Waqas et al. 2019 demonstrated that nutrient priming with a compost tea based on food waste enhanced germination, root length and seed germination index of mung beans. The authors reported that the compost used for priming contained 117.91 and 168 mg/kg of $NO_3$-N and $NH_4$-N, respectively. Frass used to prepare the extract in our study was instead characterised by a definitely lower content of nitric nitrogen ($3.02 \pm 0.01$ mg/kg) and higher ammonium nitrogen ($4770.1 \pm 0.51$ mg/kg) (see Table S1). In a study on the N- and P-fertilisation effect induced by BSF by-products (frass, larval exuviae, and dead adult flies) on maize, frass showed low N-fertilisation effects attributed to volatilisation losses, mainly driven by high pH and ammonium content (Gärttling, Kirchner, and Schulz 2020). In addition, the high ratio organic C/total N, that is 40, suggests that frass used in the current work could determine a sort of immobilisation and further low availability of nutrients (Lopes, Yong, and Lalander 2022). This could be the reason for the low efficacy of seed priming with pFE in tomato. On the contrary, primed wheat seeds were able to germinate and grow better (see Figure 4) because they probably absorbed more nutrients in presence of $NH_4$-N than $NO_3$-N, as previously reported (Spratt and Gasser 1970; Goyal and Huffaker 2015). Indeed, dry weight of seedlings derived from wheat seeds primed with pFE was almost always higher than control, in particular during the combined activity of pFE and T22 (see Figure 5).

In addition to the interesting results obtained regarding the biostimulant effect induced by pFE priming on wheat seeds, our results demonstrated a good beneficial effect against *F. sporotrichioides* because the disease incidence was reduced of 57% and 78% when used alone or combined with T22, respectively. These findings are better if compared to the observation of Quilliam et al. 2020, where the amendment with frass from BSF larvae significantly reduced by about 40% the dead plants by *Fusarium* wilt disease in cowpea. Moreover, in our work frass



2767035x, 2024, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/sae2.70030 by Patrizia Falabella - University Basilicata Di Potenza Bibl Interdepartim Di Ateneo , Wiley Online Library on [03/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

performed better also compared to the outcomes of a suppressive bioassay on garden cress damping-off caused by *Sclerotinia minor*, that was significantly reduced by BSF larvae frass of about 30% (Setti et al. 2019). Indeed, the present work represents a pioneristic and positive first evaluation on the reduction of *Fusarium*-induced pressure with the use of BSF frass in wheat, showing its ability to be also used together with the biological control agent *T. harzianum*.

After the recognition of pathogens, the plant can trigger several defence mechanisms, including the production of ROS (reactive oxygen species) and the reinforcement of cell walls, with phenolic compounds or callose deposition (Chrpová et al. 2021). The faster the induction of antioxidant production, the better is the plant's resistance to the pathogen (Khaledi, Taheri, and Falahati-Rastegar 2016).

Nonenzymatic antioxidant compounds, like phenolic compounds, can act directly in the detoxification of ROS and radicals, or they can reduce substrates for antioxidant enzymes (Caverzan, Casassola, and Patussi Brammer 2016). They have structural function in lignification and antibiotic activity and are produced in response to attack and infection of pathogens through the phenylpropanoid pathway (Orzali, Forni, and Riccioni 2014).

In our experiment, performed on durum wheat cultivar *Simeto*, highly susceptible to *Fusarium* spp (Orzali, Forni, and Riccioni 2014), the highest total phenolic content (TPC) was detected in infected seedlings, while all priming treatments did not induce significant changes in TPC, compared to control (see Figure 6). Similarly, Khaledi et al. (Khaledi, Taheri, and Falahati-Rastegar 2016) reported that two infected wheat cultivars, partially resistant and susceptible to *F. graminearum* and *F. culmorum*, respectively, led to accumulation of non enzymatic and enzymatic antioxidants compounds, and that this accumulation occurred more rapidly in the resistant cultivar. Our results confirmed those obtained by Orzali, Forni and Riccioni (2014) in a study with seed priming carried out with chitosan, that led to the increase in phenolic content in the same *Simeto* cultivar seedlings, with even higher accumulation when the pathogen *F. graminearum* was inoculated. Phenolic compounds have a broad spectrum of antifungal activity and could be conjugated to the cell wall, by cross-linking polysaccharides and between polysaccharides and lignin, to prevent pathogen colonisation and trigger an active mechanism of resistance (Chrpová et al. 2021; McKeehen 1999). Hakulinen, Sorjonen, and Julkunen-Tiitto (1999) asserted that the lowered TPC is probably due to the synthesis of lignin, a polymer of oxidised phenolic alcohols, that is bound to host plant cell walls to enforce them and impede and/or prevent the penetration of fungal hyphae (Dixon and Paiva 1995). Pastuszak et al. 2021 observed a decrease in TPC associated with a high content of cell-wall-bound phenolic compounds (CWP) in a resistant genotype to *F. culmorum*. In addition, they observed that TPC and CWP content were negatively and positively correlated with the content of $H_2O_2$, respectively. This negative correlation during *Fusarium* infection was interpreted in a TP action as antioxidants for the possible reduction in $H_2O_2$ amount. Therefore, the high TPC that we observed only in the infected seedlings (FS) and the lowered TPC induced by priming treatments with pFE (pFE vs. FS) and T22 (T22 vs. FS), more when they act alone that together (pFE + T22 vs. FS) suggests the defensive role of phenolics during fungal infection. Salycilic acid acts as an inducer of systemic acquired resistance (SAR) in plant. Its probable presence among phenolic compounds produced in *Simeto* seedlings derived from primed seeds with T22 and pFE could be the responsible for the plant's resistance to *F. sporotrichioides* attack. This SAR could be due to the possible production of pathogenesis-related proteins that act in the decomposition of the fungal hyphae cell wall (Pastuszak et al. 2021; Derckel et al. 1998; Arora et al. 2007).

ROS production and metabolism are controlled by several enzymes, especially SOD, that acts as the frontline in the defence mechanism, catalysing the dismutation of superoxide $O_2^-$ into $O_2$ and $H_2O_2$ (Caverzan, Casassola, and Patussi Brammer 2016).

In our experiments, SOD activity always significantly decreased in presence of FS, also following priming with pFE and T22, alone or combined. By contrast, the highest SOD activity was in healthy control plantlets, suggesting that this enzyme plays an important role in ROS detoxification, to limit or prevent oxidative damages caused by the fungus (Vitti et al. 2016). Our findings are partially in line with the study on *Triticum aestivum* cv. *Zyta*, treated with *T. harzianum*, that was able to control stress induced by *F. culmorum* by reducing SOD activity in 7 days old seedlings, but where the presence of the pathogen alone determined an increase in SOD activity (Mironenka, Różalska, and Bernat 2021).

## 5 | Conclusions

The study explored, for the first time, frass deriving from *H. illucens* as a new sustainable tool for inducing biostimulant and/or antifungal activities in tomato and wheat plants against *Fusarium* spp.

In particular, the well-established technique of seed priming was employed with the frass extract (pFE) as an innovative and eco-friendly formulation to stimulate seed germination and seedlings growth, as well as enhance protection against the pathogens. Results obtained by germination tests on water agar were significant to gain knowledge of the effectiveness of using priming for durum wheat (*Triticum durum* Desf. var *Simeto*), with an increased performance in seedling growth of 44% compared to untreated control, but not for tomato (*Solanum lycopersicum* L. var *Cerasiforme*). In addition, phytotoxicity and in vitro antagonism assessments allowed to define the optimal dilution of 10% pFE to effectively use also for reducing the *F. sporotrichioides*-induced disease incidence (DI) in wheat. This reduction could be due to biochemical modifications induced by priming with pFE alone (57% DI reduction), as well as combined with the known effective biocontrol agent *T. harzianum* T22 (78% DI reduction). Differences in TPCand SOD activity observed in seedlings derived from treated seeds compared to untreated control, seem to indicate that the highly susceptible to *Fusarium* spp. cultivar *Simeto* brings into play both enzymatic and nonenzymatic responses as defence mechanisms during the specific interaction with pathogen *F. sporotrichioides*. Specifically, phenolic compounds showed a pivotal role in reinforcing the host cell walls to impede and/or prevent the



2767035x, 2024, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/sae2.70030 by Patrizia Falabella - University Basilicata Di Potenza Bibl Interdepartim Di Ateneo , Wiley Online Library on [03/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

penetration of fungal hyphae. In light of overall results obtained when pFE was used together with T22 (pFE + T22 vs. FS), it is possible to conclude that they could work well in combination to obtain both biostimulation and antifungal effect. Deeper studies should be addressed to evaluate the ability of frass to induce the same responses shown here, also when used in different formulations and/or pathosystems. For practical applications in sustainable agriculture, the optimal dilution of frass extract and its combination with biological control agents like *T. harzianum* spp. could provide an effective strategy for improving crop health and yield. Therefore, our results open the door for further research in potted and field conditions for implementing frass as a green, circular economy-based and sustainable tool to be used in agriculture systems, also taking into account that the production cost of BSF frass can have a competitive advantage compared to other fertiliser/biostimulant products. A significant factor in this cost-efficiency contest is the larval ability to feed on organic by-products and waste, reducing feed input costs and offering a solution to managing organic waste, mitigating the high impact of conventional waste disposal. In addition, life cycle assessment studies demonstrated that BSF farms have a low environmental footprint in terms of greenhouse gas emissions, land and water use, giving an added value to the production process and fostering its economic and environmental-friendly feasibility.

## Author Contributions

Conceptualisation: Leonardo Coviello, Maria Nuzzaci, Patrizia Falabella, and Antonella Vitti. Methodology: Leonardo Coviello, Maria Nuzzaci, and Antonella Vitti. Formal analysis: Leonardo Coviello and Antonella Vitti. Investigation: Leonardo Coviello and Antonella Vitti. Resources: Patrizia Falabella and Maria Nuzzaci. Data curation: Leonardo Coviello, Maria Nuzzaci, Antonella Vitti, Patrizia Falabella, Carmen Scieuzo, Rosanna Salvia, and Domenico Ronga. Writing–original draft preparation: Leonardo Coviello, Maria Nuzzaci, and Antonella Vitti. Writing–review and editing: Leonardo Coviello, Maria Nuzzaci, Antonella Vitti, Patrizia Falabella, Carmen Scieuzo, Rosanna Salvia, and Domenico Ronga. Supervision: Maria Nuzzaci and Antonella Vitti. Validation: Leonardo Coviello, Maria Nuzzaci, and Antonella Vitti. Project administration: Leonardo Coviello, Maria Nuzzaci, and Antonella Vitti. Funding acquisition: Maria Nuzzaci and Patrizia Falabella. All authors have read and agreed to the published version of the manuscript.


## Acknowledgements

This study was carried out within the Agritech National Research Center and received funding from the European Union Next- GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR)—MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4—D.D. 1032 17/06/2022, CN00000022). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them. Open access publishing facilitated by Universita degli Studi della Basilicata, as part of the Wiley - CRUI-CARE agreement.


## Conflicts of Interest

The authors declare no conflicts of interest.

## Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## References


Alisaac, E., and A.-K. Mahlein. 2023. "Fusarium Head Blight on Wheat: Biology, Modern Detection and Diagnosis and Integrated Disease Management." *Toxins* 15: 192.

Arabzadeh, G., M. Delisle-Houde, R. J. Tweddell, et al. 2022. "Diet Composition Influences Growth Performance, Bioconversion of Black Soldier Fly Larvae: Agronomic Value and In Vitro Biofungicidal Activity of Derived Frass." *Agronomy* 12: 1765.

Arora, N. K., M. J. Kim, S. C. Kang, and D. K. Maheshwari. 2007. "Role of Chitinase and β-1,3-Glucanase Activities Produced by a Fluorescent Pseudomonad and In Vitro Inhibition of *Phytophthora Capsici* and *Rhizoctonia Solani*." *Canadian Journal of Microbiology* 53: 207–212.

Bargaz, A., K. Lyamlouli, M. Chtouki, Y. Zeroual, and D. Dhiba. 2018. "Soil Microbial Resources for Improving Fertilizers Efficiency in an Integrated Plant Nutrient Management System." *Frontiers in Microbiology* 9: 1606.

Barragán-Fonseca, K. Y., L. O. Greenberg, G. Gort, M. Dicke, and J. J. A. Van Loon. 2023. "Amending Soil With Insect Exuviae Improves Herbivore Tolerance, Pollinator Attraction and Seed Yield of *Brassica Nigra* Plants." *Agriculture, Ecosystems & Environment* 342: 108219.

Beesigamukama, D., B. Mochoge, N. K. Korir, et al. 2020. "Exploring Black Soldier Fly Frass as Novel Fertilizer for Improved Growth, Yield, and Nitrogen Use Efficiency of Maize Under Field Conditions." *Frontiers in Plant Science* 11: 574592.

Bochicchio, R., R. Labella, A. Vitti, M. Nuzzaci, G. Logozzo, and M. Amato. 2022. "Root Morphology, Allometric Relations and Rhizosheath of Ancient and Modern Tetraploid Wheats (*Triticum durum* Desf.) in Response to Inoculation With *Trichoderma harzianum* T-22." *Plants* 11: 159.

Bohm, K., G. A. Hatley, B. H. Robinson, and M. J. Gutiérrez-Ginés. 2023. "Analysis of Chemical and Phytotoxic Properties of Frass Derived From Black Soldier Fly-Based Bioconversion of Biosolids." *Sustainability* 15: 11526.

Boudabbous, K., S. B. M. Hammami, W. Toukabri, et al. 2023. "Black Soldier Fly (*Hermetia illucens*) Larvae Frass Organic Fertilizer Improves Soil Quality and the Productivity of Durum Wheat." *Communications in Soil Science and Plant Analysis* 54: 2491–2507.

Carroll, A., M. Fitzpatrick, and S. Hodge. 2023. "The Effects of Two Organic Soil Amendments, Biochar and Insect Frass Fertilizer, on Shoot Growth of Cereal Seedlings." *Plants* 12: 1071.

Caverzan, A., A. Casassola, and S. Patussi Brammer. 2016. "Reactive Oxygen Species and Antioxidant Enzymes Involved in Plant Tolerance to Stress." In *Abiotic and Biotic Stress in Plants - Recent Advances and Future Perspectives*, edited by A. K. Shanker and C. Shanker. InTech. https://doi.org/10.5772/61368.

Chandra Nayaka, S., S. R. Niranjana, A. C. Uday Shankar, et al. 2010. "Seed Biopriming With Novel Strain of *Trichoderma harzianum* for the Control of Toxigenic *Fusarium verticillioides* and Fumonisins in Maize." *Archives of Phytopathology and Plant Protection* 43: 264–282.

Chaverri, P., F. Branco-Rocha, W. Jaklitsch, R. Gazis, T. Degenkolb, and G. J. Samuels. 2015. "Systematics of the *Trichoderma harzianum* Species Complex and the Re-Identification of Commercial Biocontrol Strains." *Mycologia* 107: 558–590.

Chrpová, J., M. Orsák, P. Martinek, J. Lachman, and M. Trávníčková. 2021. "Potential Role and Involvement of Antioxidants and Other Secondary Metabolites of Wheat in the Infection Process and Resistance to Fusarium spp." *Agronomy* 11: 2235.

Chun, S.-C., and M. Chandrasekaran. 2019. "Chitosan and Chitosan Nanoparticles Induced Expression of Pathogenesis-Related Proteins Genes Enhances Biotic Stress Tolerance in Tomato." *International Journal of Biological Macromolecules* 125: 948–954.

Commission Regulation. (2011). (EU) No 142/2011. *Commission Regulation (EU) No 142/2011 of 25 February 2011 Implementing Regulation*





2767035x, 2024, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/sae2.70030 by Patrizia Falabella - University Basilicata Di Potenza Bibl Interdepartim Di Ateneo , Wiley Online Library on [03/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

*(EC) No 1069/2009 of the European Parliament and of the Council Laying Down Health Rules as Regards Animal By-Products and Derived Products Not Intended for Human Consumption and Implementing Council Directive 97/78/EC as Regards Certain Samples and Items Exempt From Veterinary Checks at the Border Under that DirectiveText With EEA Relevance* OJ L 54, 1.

Cretoiu, M. S., G. W. Korthals, J. H. M. Visser, and J. D. Van Elsas. 2013. "Chitin Amendment Increases Soil Suppressiveness Toward Plant Pathogens and Modulates the Actinobacterial and Oxalobacteraceal Communities in an Experimental Agricultural Field." *Applied and Environmental Microbiology* 79: 5291–5301.

Dawd, S. M., and S. S. Abdulla. 2020. "Effect of Different Salt Concentrations on Ratio, Speed, Growth and Development of Seedlings of Some Vegetable Crops." *International Journal of Agricultural and Statistical Sciences* 16: 1755–1759.

Derckel, J. P., J. C. Audran, B. Haye, B. Lambert, and L. Legendre. 1998. "Characterization, Induction by Wounding and Salicylic Acid, and Activity Against *Botrytis cinerea* of Chitinases and β-1,3-Glucanases of Ripening Grape Berries." *Physiologia Plantarum* 104: 56–64.

Dixon, R. A., and N. L. Paiva. 1995. "Stress-Induced Phenylpropanoid Metabolism." *The Plant Cell* 7: 1085–1097.

Ekwomadu, T. I., and M. Mwanza. 2023. "Fusarium Fungi Pathogens, Identification, Adverse Effects, Disease Management, and Global Food Security: A Review of the Latest Research." *Agriculture (London)* 13: 1810.

Elissen, H., M. Schilder, J. Postma, and R. Van Der Weide. 2019. Disease Suppression in Cress and Sugar Beet Seedlings With Frass of the Black Soldier Fly (*Hermetia illucens*). https://research.wur.nl/en/publications/46d55a98-db1e-4144-8545-04411cd756b4. https://doi.org/10.18174/515047.

European Commission. 2021. *Regulation (EU) No 2021/1925 of 5 November 2021 Amending Certain Annexes to Regulation (EU) No 142/2011 as Regards the Requirements for Placing on the Market of Certain Insects Products and the Adaptation of a Containment Method.*

Ferrigo, D., M. Mondin, E. Ladurner, F. Fiorentini, R. Causin, and A. Raiola. 2020. "Effect of Seed Biopriming With Trichoderma harzianum Strain INAT11 on Fusarium Ear Rot and Gibberella Ear Rot Diseases." *Biological Control* 147: 104286.

Food and Agriculture Organization of United Nations. Crops and Livestock Products. 2023.

Francesconi, S., B. Steiner, H. Buerstmayr, M. Lemmens, M. Sulyok, and G. M. Balestra. 2020. "Chitosan Hydrochloride Decreases Fusarium Graminearum Growth and Virulence and Boosts Growth, Development and Systemic Acquired Resistance in Two Durum Wheat Genotypes." *Molecules* 25: 4752.

Franco, A., R. Rinaldi, F. Giglio, et al. 2024. "Edible Insects: An Overview on Farming, From Processing Procedures to Environmental Impact, With a Glimpse to Traditional Recipes and to Future Cultured Meat." *Entomologia Generalis* 44, no. 4: 813–831. https://doi.org/10.1127/entomologia/2024/2651.

Francois, L. E., E. V. Maas, T. J. Donovan, and V. L. Youngs. 1986. "Effect of Salinity on Grain Yield and Quality, Vegetative Growth, and Germination of Semi-Dwarf and Durum Wheat." *Agronomy Journal* 78: 1053–1058.

Fuhrmann, A., B. Wilde, R. F. Conz, et al. 2022. "Residues From Black Soldier Fly (*Hermetia illucens*) Larvae Rearing Influence the Plant-Associated Soil Microbiome in the Short Term." *Frontiers in Microbiology* 13: 994091.

Gärttling, D., S. M. Kirchner, and H. Schulz. 2020. "Assessment of the N- and P-Fertilization Effect of Black Soldier Fly (Diptera: Stratiomyidae) By-Products on Maize." *Journal of Insect Science* 20, no. 8. https://doi.org/10.1093/jisesa/ieaa089.

Gebremikael, M. T., A. Ranasinghe, P. S. Hosseini, et al. 2020. "How Do Novel and Conventional Agri-Food Wastes, Co-Products and By-Products Improve Soil Functions and Soil Quality?" *Waste Management* 113: 132–144.

Geng, W., Y. Zhao, Z. Mao, X. Wang, N. Wu, and X. Xu. 2022. "The Effects of Combined Use of Black Soldier Fly Larvae Frass Fertilizer With Exogenous Selenium on Rice Growth and Accumulation of Heavy Metals." *Journal of Soil Science and Plant Nutrition* 22: 5133–5143.

Godlewska, K., D. Ronga, and I. Michalak. 2021. "Plant Extracts - Importance in Sustainable Agriculture." *Italian Journal of Agronomy* 16. https://doi.org/10.4081/ija.2021.1851.

Góral, T., and E. Arseniuk. 2006. "Pathogenicity and Resistance in Fusarium Spp.-Wheat, Triticale and Rye Pathosystems at the Seedling Stage." *Plant Breeding and Seed Science* 54: 3–15.

Goyal, S. S., and R. C. Huffaker. 2015. "Nitrogen Toxicity in Plants." In *ASA, CSSA, and SSSA Books*, edited by R. D. Hauck, 97–118. Madison, WI, USA: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America. https://doi.org/10.2134/1990.nitrogenincropproduction.c6.

Graves, S., H.-P. Piepho, and L. Selzer. multcompView: Visualizations of Paired Comparisons. 2024.

Hakulinen, J., S. Sorjonen, and R. Julkunen-Tiitto. 1999. "Leaf Phenolics of Three Willow Clones Differing in Resistance to Melampsora Rust Infection." *Physiologia Plantarum* 105: 662–669.

Hogsette, J. A. 1992. "New Diets for Production of House Flies and Stable Flies (Diptera: Muscidae) in the Laboratory." *Journal of Economic Entomology* 85: 2291–2294.

Iqbal, N., R. Riyazuddin, M. Nauman, et al. 2024. "Role of Plant Defence System in Crop Protection Against Fusarium Pathogens." In *Fusarium - Recent Studies*, edited by I. Y. Abdurakhmonov. IntechOpen. https://doi.org/10.5772/intechopen.1004924.

Kawasaki, K., T. Kawasaki, H. Hirayasu, Y. Matsumoto, and Y. Fujitani. 2020. "Evaluation of Fertilizer Value of Residues Obtained After Processing Household Organic Waste With Black Soldier Fly Larvae (*Hermetia illucens*)." *Sustainability* 12: 4920.

Khaledi, N., P. Taheri, and M. Falahati-Rastegar. 2016. "Reactive Oxygen Species and Antioxidant System Responses in Wheat Cultivars During Interaction With Fusarium Species." *Australasian Plant Pathology* 45: 653–670.

Kubiak, A., A. Wolna-Maruwka, A. A. Pilarska, A. Niewiadomska, and A. Piotrowska-Cyplik. 2023. "Fungi of the Trichoderma Genus: Future Perspectives of Benefits in Sustainable Agriculture." *Applied Sciences* 13: 6434.

Labella, R., R. Bochicchio, R. Addesso, et al. 2024. "Germination Behavior and Geographical Information System-Based Phenotyping of Root Hairs to Evaluate the Effects of Different Sources of Black Soldier Fly (*Hermetia illucens*) Larval Frass on Herbaceous Crops." *Plants* 13: 230.

Lindgren, E., F. Harris, A. D. Dangour, et al. 2018. "Sustainable Food Systems—A Health Perspective." *Sustainability Science* 13: 1505–1517.

Lomonaco, G., A. Franco, J. De Smet, C. Scieuzo, R. Salvia, and P. Falabella. 2024. "Larval Frass of *Hermetia illucens* as Organic Fertilizer: Composition and Beneficial Effects on Different Crops." *Insects* 15: 293.

Lopes, I. G., J. W. Yong, and C. Lalander. 2022. "Frass Derived From Black Soldier Fly Larvae Treatment of Biodegradable Wastes. A Critical Review and Future Perspectives." *Waste Management* 142: 65–76.

Lu, J., Y. Guo, A. Muhmood, et al. 2022. "Probing the Antioxidant Activity of Functional Proteins and Bioactive Peptides in *Hermetia illucens* Larvae Fed With Food Wastes." *Scientific Reports* 12: 2799.

Luo, Y., J. Liang, G. Zeng, et al. 2018. "Seed Germination Test for Toxicity Evaluation of Compost: Its Roles, Problems and Prospects." *Waste Management* 71: 109–114.



2767035x, 2024, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/sae2.70030 by Patrizia Falabella - University Basilicata Di Potenza Bibl Interdepartim Di Ateneo , Wiley Online Library on [03/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Mahmood, A., and R. Kataoka. 2019. "Application of Endophytes Through Seed Priming." In *Priming and Pretreatment of Seeds and Seedlings*, edited by M. Hasanuzzaman and V. Fotopoulos, 509–521. Singapore: Springer Singapore. https://doi.org/10.1007/978-981-13-8625-1_25.

Manjula, K., and A. R. Podile. 2005. "Increase in Seedling Emergence and Dry Weight of Pigeon Pea in the Field With Chitin-Supplemented Formulations of Bacillus Subtilis AF 1." *World Journal of Microbiology and Biotechnology* 21: 1057–1062. https://doi.org/10.1007/s11274-004-8148-z.

McKeehen, J. D., R. H. Busch, and R. G. Fulcher. 1999. "Evaluation of Wheat (*Triticum aestivum* L.) Phenolic Acids During Grain Development and Their Contribution to Fusarium Resistance." *Journal of Agricultural and Food Chemistry* 47, no. 4: 1476–1482. https://pubs.acs.org/doi/full/10.1021/jf980896f.

Mironenka, J., S. Różalska, and P. Bernat. 2021. "Potential of Trichoderma Harzianum and Its Metabolites to Protect Wheat Seedlings Against Fusarium Culmorum and 2,4-D." *International Journal of Molecular Sciences* 22: 13058.

Nazari, L., E. Pattori, S. Somma, et al. 2019. "Infection Incidence, Kernel Colonisation, and Mycotoxin Accumulation in Durum Wheat Inoculated With Fusarium Sporotrichioides, F. Langsethiae or F. Poae at Different Growth Stages." *European Journal of Plant Pathology* 153: 715–729.

Orzali, L., C. Forni, and L. Riccioni. 2014. "Effect of Chitosan Seed Treatment as Elicitor of Resistance to Fusarium Graminearum in Wheat." *Seed Science and Technology* 42: 132–149.

Pastuszak, J., A. Szczerba, M. Dziurka, et al. 2021. "Physiological and Biochemical Response to Fusarium Culmorum Infection in Three Durum Wheat Genotypes at Seedling and Full Anthesis Stage." *International Journal of Molecular Sciences* 22: 7433.

Poveda, J. 2021. "Insect Frass in the Development of Sustainable Agriculture. A Review." *Agronomy for Sustainable Development* 41: 5.

Quilliam, R. S., C. Nuku-Adeku, P. Maquart, D. Little, R. Newton, and F. Murray. 2020. "Integrating Insect Frass Biofertilisers Into Sustainable Peri-Urban Agro-Food Systems." *Journal of Insects as Food and Feed* 6: 315–322.

Rachitha, P., K. Krupashree, G. Jayashree, N. Gopalan, and F. Khanum. 2017. "Growth Inhibition and Morphological Alteration of Fusarium Sporotrichioides by *Mentha Piperita* Essential Oil." *Pharmacognosy Research* 9: 74.

Romano, N., S. N. Datta, A. K. Sinha, and G. S. J. Pande. 2023. "Partially Replacing Synthetic Fertilizer With Black Soldier Fly (*Hermetia illucens*) Larvae Frass Enhances Kale (*Brassica oleracea* Var. Sabellica) Production." *Technology in Horticulture* 3, no. 1.

Ronga, D., A. Vitti, M. Zaccardelli, et al. 2021. "Root Zone Management for Improving Seedling Quality of Organically Produced Horticultural Crops." *Agronomy* 11: 630.

Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012. "NIH Image to ImageJ: 25 Years of Image Analysis." *Nature Methods* 9: 671–675.

Scieuzo, C., A. Franco, R. Salvia, et al. 2023. "Enhancement of Fruit Byproducts Through Bioconversion by *Hermetia illucens* (Diptera: Stratiomyidae)." *Insect Science* 30: 991–1010.

Sempere Ferre, F., and M. P. Santamarina. 2010. "Efficacy of *Trichoderma harzianum* in Suppression of Fusarium Culmorum." *Annals of Microbiology* 60: 335–340.

Setlow, P. 2016. "Spore Resistance Properties." In *The Bacterial Spore*, edited by A. Driks and P. Eichenberger, 201–215. Washington, DC, USA: ASM Press. https://doi.org/10.1128/9781555819323.ch10.

Setti, L., E. Francia, A. Pulvirenti, et al. 2019. "Use of Black Soldier Fly (*Hermetia illucens* (L.), Diptera: Stratiomyidae) Larvae Processing Residue in Peat-Based Growing Media." *Waste Management* 95: 278–288.

Sher, A., T. Sarwar, A. Nawaz, M. Ijaz, A. Sattar, and S. Ahmad. 2019. "Methods of Seed Priming." In *Priming and Pretreatment of Seeds and Seedlings*, edited by M. Hasanuzzaman and V. Fotopoulos, 1–10. Singapore: Springer. https://doi.org/10.1007/978-981-13-8625-1_1.

Simões, D., and E. De Andrade. 2023. "Fusarium Species Responsible for Tomato Diseases and Mycotoxin Contamination and Biocontrol Opportunities." In *Fusarium - Recent Studies*, edited by I. Y. Abdurakhmonov. IntechOpen. https://doi.org/10.5772/intechopen.1003643.

Sood, M., D. Kapoor, V. Kumar, et al. 2020. "Trichoderma: The 'Secrets' of a Multitalented Biocontrol Agent." *Plants* 9: 762.

Spratt, E. D., and J. K. R. Gasser. 1970. "Effects of Fertilizer-Nitrogen and Water Supply on Distribution of Dry Matter and Nitrogen Between the Different Parts of Wheat." *Canadian Journal of Plant Science* 50: 613–625.

Srinivas, C., D. Nirmala Devi, K. Narasimha Murthy, et al. 2019. "Fusarium Oxysporum f. sp. Lycopersici Causal Agent of Vascular Wilt Disease of Tomato: Biology to Diversity– A Review." *Saudi Journal of Biological Sciences* 26: 1315–1324.

Sui, L., J. Li, J. Philp, et al. 2022. "Trichoderma Atroviride Seed Dressing Influenced the Fungal Community and Pathogenic Fungi in the Wheat Rhizosphere." *Scientific Reports* 12: 9677.

Surendra, K. C., J. K. Tomberlin, A. van Huis, J. A. Cammack, L. H. L. Heckmann, and S. K. Khanal. 2020. "Rethinking Organic Wastes Bioconversion: Evaluating the Potential of the Black Soldier Fly (*Hermetia illucens* (L.)) (Diptera: Stratiomyidae) (BSF)." *Waste Management* 117: 58–80.

Triunfo, M., E. Tafi, A. Guarnieri, et al. 2022. "Characterization of Chitin and Chitosan Derived From *Hermetia illucens*, a Further Step in a Circular Economy Process." *Scientific Reports* 12: 6613.

Tsolakidou, M.-D., G. Demetriou, S. Panagiotou, L. Vassiliou, V. Goulas, and I. Pantelides. 2024. "Efficacy of Commercial Biocontrol Products for the Management of Verticillium and Fusarium Wilt in Greenhouse Tomatoes: Impact on Disease Severity, Fruit Yield, and Quality." *Agriculture* 14: 882.

Uwineza, P. A., M. Urbaniak, Ł. Stępień, A. Gramza-Michałowska, and A. Waśkiewicz. 2024. "Efficacy of *Lamium Album* as a Natural Fungicide: Impact on Seed Germination, Ergosterol, and Mycotoxins in Fusarium Culmorum-Infected Wheat Seedlings." *Frontiers in Microbiology* 15: 1363204.

Veeken, A. H. M., W. J. Blok, F. Curci, G. C. M. Coenen, A. J. Termorshuizen, and H. V. M. Hamelers. 2005. "Improving Quality of Composted Biowaste to Enhance Disease Suppressiveness of Compost-Amended, Peat-Based Potting Mixes." *Soil Biology and Biochemistry* 37: 2131–2140.

Vitti, A., V. Bevilacqua, G. Logozzo, R. Bochicchio, M. Amato, and M. Nuzzaci. 2022. "Seed Coating With *Trichoderma harzianum* T-22 of Italian Durum Wheat Increases Protection Against Fusarium Culmorum-Induced Crown Rot." *Agriculture* 12: 714.

Vitti, A., L. Coviello, M. Nuzzaci, et al. 2024. "Biostimulation of Humic Acids on *Lepidium sativum* L. Regulated by Their Content of Stable Phenolic O· Radicals." *Chemical and Biological Technologies in Agriculture* 11: 92.

Vitti, A., E. Pellegrini, C. Nali, et al. 2016. "*Trichoderma harzianum* T-22 Induces Systemic Resistance in Tomato Infected by Cucumber Mosaic Virus." *Frontiers in Plant Science* 7. https://doi.org/10.3389/fpls.2016.01520.

Vitti, A., A. Sofo, A. Scopa, and M. Nuzzaci. 2015. "Sustainable Agricultural Practices in Disease Defence of Traditional Crops in Southern Italy: The Case Study of Tomato Cherry Protected by *Trichoderma harzianum* T-22 Against Cucumber Mosaic Virus (CMV)." *The Sustainability of Agro-Food and Natural Resource Systems in the Mediterranean Basin*: 133–143.



27670355x, 2024, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/sae2.70030 by Patrizia Falabella - University Basilicata Di Potenza Bibl Interdepartim Di Ateneo , Wiley Online Library on [03/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Waqas, M., N. E. Korres, M. D. Khan, et al. 2019. "Priming and Pretreatment of Seeds and Seedlings." In *Advances in the Concept and Methods of Seed Priming*, edited by M. Hasanuzzaman and V. Fotopoulos, 11–41. Singapore: Springer. https://doi.org/10.1007/978-981-13-8625-1_2.

Wickham, H., M. Averick, J. Bryan, et al. 2019. "Welcome to the Tidyverse." *Journal of Open Source Software* 4: 1686.

Wiśniewska, H., T. Basiński, J. Chełkowski, and J. Perkowski. 2011. "Fusarium Sporotrichioides Sherb. Toxins Evaluated in Cereal Grain With Trichoderma harzianum." *Journal of Plant Protection Research* 51: 134–139.

Yildirim, E., M. Ekinci, T. Günaydin, E. Güven, and H. İ. Öztürk. 2022. "How Salinity Affect Emergence of Garden Cress (*Lepidium sativum* L.) Cultivars?" *Erciyes Tarım ve Hayvan Bilimleri Dergisi* 5: 38–43.

## Supporting Information

Additional supporting information can be found online in the Supporting Information section.



2767035x, 2024, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/sae2.70030 by Patrizia Falabella - University Basilicata Di Potenza Bibl Interdepartim Di Ateneo , Wiley Online Library on [03/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License